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Research Focus

Matrix metalloproteinases in reproductive endocrinology

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One of the most common mechanisms for transactivation of epidermal growth factor receptor (EGF-R) by G protein-coupled receptors (GPCRs) is through the release of local EGF-like ligands from transmembrane precursors by the proteolytic action of matrix metalloproteinases (MMPs). These enzymes are crucial factors in the normal physiology of the reproductive system and also participate in neuroendocrine regulation through mediation of gonadotropin-releasing hormone (GnRH) action. Recent studies by Roelle *et al.* showed that GnRH-induced activation of the EGF-R and extracellular signal-regulated kinases 1 and 2 (ERK1/2) in pituitary gonadotrophs occurs through ectodomain shedding of heparin binding-EGF (HB-EGF) by MMP2 and MMP9, indicating a crucial role for MMPs in GnRH signaling.

The extracellular matrix (ECM) is crucial for providing an environment for cell migration, division, differentiation, anchorage and survival or death. The highly regulated control of ECM homeostasis is governed, in part, by the action of a specific class of proteolytic enzymes known as matrix metalloproteinases (MMPs), a group of zinc- and calcium-dependent enzymes. MMPs are synthesized as zymogens with a signal sequence and a propeptide segment that is removed during activation. Once activated, some MMPs are secreted into the ECM, whereas others, the membrane type (MT)-MMPs, remain tethered to the cell surface by a hydrophobic transmembrane domain. The main function of MMPs seems to be tissue remodeling, and they are involved in a variety of processes including wound healing, embryonic development, nerve growth, angiogenesis and the release of defense proteins and growth factors. Under normal conditions, their proteolytic activity is precisely regulated by their endogenous protein inhibitors, termed tissue-specific inhibitors of MMPs (TIMPs). Disruption of the balance between MMPs and TIMPs contributes to pathophysiological processes such as arthritis and tumor growth and metastasis [1,2].

Role of MMPs in reproductive function

Substantial evidence supports a general role for MMPs in the control of many aspects of reproductive function that require tissue remodeling and cell growth. For example, the ovary and uterus undergo extensive tissue remodeling accompanied by cyclic hormonal changes with each estrous or menstrual cycle. In the ovary, tissue remodeling is required for the growth and expansion of the follicle, breakdown of the follicular wall before ovulation, transformation of the post-ovulatory follicle into the corpus luteum and structural dissolution of the latter during luteal regression. Similarly, there is extensive turnover of the endometrial connective tissue matrix and its regrowth during each menstrual cycle. Hormonal signals in the ovary and uterus modulate individual MMPs and TIMPs to control the type of matrix to be remodeled, the site-specific location and the extent of degradation [2].

Transactivation of the epidermal growth factor receptor

MMPs have been implicated in the control of membrane fusion, cytokine and growth factor shedding and cell migration, as well as processes such as muscle development, fertilization and cell-fate determination. Many ligands for the epidermal growth factor (EGF) receptor family are shed from cell surfaces in response to specific signals. One such ligand is heparin-binding EGF (HB-EGF), which is processed in response to activation of protein kinase C (PKC). The protein precursor of HB-EGF contains a signal peptide, as well as heparin-binding, EGF-like, transmembrane and cytoplasmic domains. The processed HB-EGF liberated by MMP action causes phosphorylation of the EGF-R (EGF-R transactivation) and initiates downstream Ras-dependent signaling leading to activation of mitogen-activated protein kinases (MAPKs) [3]. Several recent studies have demonstrated that blockade of MMPs and ADAMs with pharmacological inhibitors or with their dominant negative mutants abolishes EGF-R transactivation, MAPK phosphorylation and hypertrophic responses in response to stimulation with various G protein-coupled receptor (GPCR) agonists [4–7]. Recently, elegant experiments by Roelle *et al.* [8] showed that transactivation of the EGF-R and extracellular signal-regulated kinase 1 and 2 (ERK1/2) phosphorylation by gonadotropin-releasing hormone (GnRH) occur through selective activation of two gelatinases, MMP2 and MMP9, in αT3–1 and LßT2 pituitary gonadotrophs [8].

GnRH-mediated ERK1/2 activation in hypothalamic neurons and pituitary gonadotrophs is MMP-dependent

The hypothalamic GnRH is a primary regulatory factor in the neuroendocrine control of reproduction and is released in an episodic manner from the hypothalamic GnRH neurons. The pulsatile delivery of GnRH to the anterior pituitary gland is essential for maintaining the circulating gonadotropin profiles that are necessary for normal

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reproductive function. In addition to regulating pituitary gonadotropin release, GnRH has extra-pituitary actions in neural and non-neural tissues and in several types of tumor cells. Immortalized GnRH-producing neurons (GT1–7 neurons) express several GPCRs and retain many of the characteristics of the native GnRH neurons, including the ability to maintain pulsatile GnRH release [9]. Recent evidence suggests that the autocrine action of GnRH on hypothalamic GnRH neurons is involved in the mechanism of pulsatile GnRH secretion. The GnRH pulses secreted from the median eminence stimulate anterior pituitary gonadotrophs and regulate the synthesis and secretion of the gonadotrophins luteinizing hormone (LH) and follicular stimulating hormone (FSH), which are primary regulators of reproductive function [10]. The stimulation of gonadotrophin synthesis and secretion by GnRH is dependent on ERK1/2 activation [11]. Recently, GnRH was also shown to have an integral role in human embryo implantation, because of its ability to regulate the balance between MMP and TIMP expression in decidual cells [12].

GnRH-mediated ERK1/2 activation in GT1–7 hypothalamic neurons occurs through the sequential activation of PKC, MMPs and EGF receptor transactivation [13,14]. Furthermore, GnRH action in αT3–1 pituitary gonadotrophs also occurs through EGF-R activation [15] but the types of MMPs involved in GnRH action were not known until recently. Roelle *et al.* [8] demonstrated that pharmacological inhibition of gelatinases by selective inhibitors R2028–2653 or treatment with ribozymes directed against MMP2 and MMP9 specifically blocked non-receptor tyrosine kinase Src and EGF receptor activation by GnRH, indicating the crucial role of gelatinase MMPs during GnRH signaling. These effects of GnRH in gonadotrophs were primarily PKC-dependent, as recently observed in GT1–7 cells [13,14,16]. Agonist-induced activation of MMPs resulted in release of HB-EGF, which caused phosphorylation of the EGF-R through activation of Src, as well as ERK-dependent induction of transcription factors c-fos and c-jun, in an ERK-dependent manner (Figure 1). By contrast, EGF-mediated ERK1/2 activation was independent of Src. Because HB-EGF action is mediated by EGF-R activation, the differential involvement of Src in ERK1/2 activation by HB-EGF and EGF needs further exploration. Taken together, these observations suggest that MMPs have a crucial role in the regulation of neuroendocrine and reproductive functions and because MMP activity is closely regulated by TIMPs, further studies are required to determine the type of TIMP(s) involved in this cascade.

In view of the fact that GnRH secretion is modulated by inputs from several GPCRs in the hypothalamus [17], it will be interesting to examine the extent to which individual GPCRs contribute to the activation of MMPs. Several recent reports have shown the crucial involvement of ADAMs 10, 12 and 17 in GPCR-mediated transactivation of the EGF-R and subsequent hypertrophic responses [7]. However, Roelle *et al.* [8] found no role for ADAMs (a disintegrin and metalloproteinase) in this cascade in αT3–1 cells. Both hypothalamus and pituitary gland express several MMPs (other than gelatinases) that are activated by PKC. Elucidation of the factors determining the specificity of gelatinase (MMP2 and MMP9) activation by GnRH awaits further investigation.

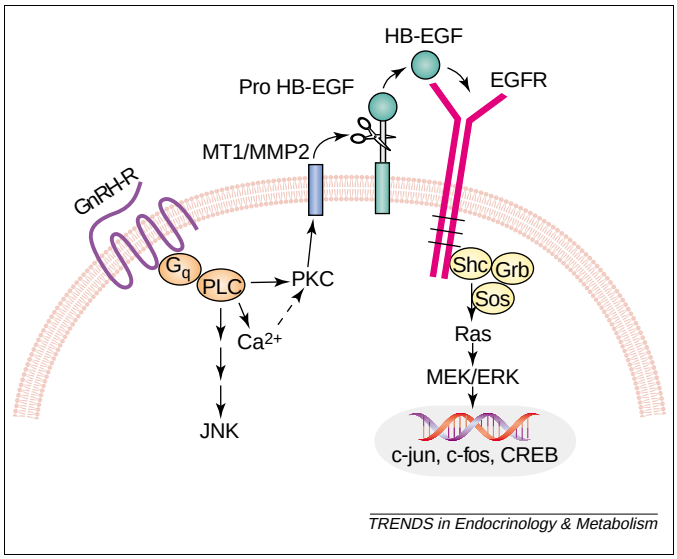

Figure 1. Signaling pathways activated by GnRH in neuroendocrine tissues. Binding of GnRH to its heptahelical receptor causes activation of PLC and generation of diacylglycerol and inositol triphosphate, which, in turn, cause activation of PKC and release of Ca^{+2} from intracellular stores. PKC activates MMP2 and MMP9, which leads to processing of proHB-EGF to HB-EGF. This phosphorylates the EGF receptor leading to activation of ERK1/2 through recruitment of Src, Grb, Sos and the activation of Ras, Raf and MEK1/2. Agonist-activated ERK1/2 and/or its dependent protein, P90 ribosomal S6 kinase, translocates to the nucleus and causes transcriptional changes including expression of c-fos, c-jun and CREB. Abbreviations: CREB, cAMP response element binding protein; EGF, epidermal growth factor; EGF-R, epidermal growth factor-receptor; ERK, extracellular signal-regulated kinase; GnRH, gonadotropin-releasing hormone; GnRH-R, gonadotropin-releasing hormone-receptor; Gq, pertassis toxin insensitive G protein; HB-EGF, heparin-binding EGF; JNK, N-terminal Jun kinase; MEK, ERK kinase; MMP, metalloproteinases; MT1, membrane type 1 MMP; PLC, phospholipase C; PKC, protein kinase C; Ras, monomeric GTPase.

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Does bone resorption inhibition affect the anabolic response to parathyroid hormone?

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One of the questions arising from the use of parathyroid hormone (PTH) as an anabolic agent is whether preventing bone loss with inhibitors of bone resorption might result in a greater amount of bone in response to PTH. Two recent independent reports indicate that the anabolic effect of PTH in osteoporotic women appears to be significantly reduced when alendronate is administered in combination with PTH in studies using biochemical markers, quantitative computed tomography and bone mineral density (BMD) measurements. The very different mechanisms of the two treatments reduces the impact of the BMD data, but if the overall conclusion is correct, an effect of PTH on resorption might be necessary for the anabolic effect to follow.

Undoubtedly the best-characterized effects of parathyroid hormone (PTH) are those that reflect its ability to stimulate bone resorption, promoting osteoclast formation *in vitro* by acting on cells of the osteoblast lineage to increase production of RANK ligand (RANKL), stimulating bone resorption in organ cultures and raising the blood calcium and increasing bone resorption *in vivo*. It might have come as a surprise to many when PTH emerged as the first, and most effective skeletal anabolic therapy when a major double-blind placebo-controlled trial of PTH(1–34) was carried out, demonstrating the efficacy of PTH in reducing vertebral fractures by 65% and non-vertebral fractures by 53% in women with osteoporosis [1]. Accumulating evidence from animal and clinical studies indicates that this is because PTH increases the formation of bone tissue, both trabecular and cortical.

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The main issue raised by the use of PTH as an anabolic agent is whether preventing bone loss with inhibitors of bone resorption might result in a greater amount of bone in response to PTH. This was addressed recently by Black *et al.* [2] and Finkelstein *et al.* [3] in two separate studies, but the clinical experiments were compromised by the available techniques. They hypothesized that giving the two treatments together would increase bone mineral density (BMD) more than either one alone. They report that concurrently administered alendronate and PTH result in inhibition of the response to PTH as assessed by computed tomography (CT) and biochemical markers. The same was true for BMD, but it is difficult to see how the BMD data can be interpreted at all.

PTH and bisphosphonates produce their effects on BMD in very different ways; PTH produces new bone tissue, which is initially under-mineralized, whereas alendronate maintains the same amount of bone tissue that undergoes more complete secondary mineralization because of the suppression of the bone-remodelling rate. It is entirely predictable that the effects are not additive, and it is therefore particularly difficult to interpret a small apparent decreased effect of combined treatment, as is seen in the data from Finkelstein *et al.* [3]. BMD measurements are easily obtained, but apart from their use in the diagnosis of osteoporosis, they can be quite misleading in evaluating the response to therapy with resorption inhibitors, in which the magnitude of effect on BMD bears little relationship to anti-fracture efficacy [4]. It is not advisable to use BMD to compare the effects of anabolic agents with those of anti-resorptive ones. Attenuation of the anabolic response to PTH by alendronate might be the conclusion drawn from CT and biochemical marker data,